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RESEARCH ARTICLE

Color Stability of Composites After Short-term Oral Simulation: An *in vitro* Study

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Abstract:

Background:

Although most of the studies investigated color stability of different restorative materials, evaluation of color stability of composites after immersion in multiple beverages in the same day by an *in vitro* oral simulation study is unclear.

Objective:

To assess color change of different restorative materials at the end of days 1, 14, and 30 of immersion in multiple liquid types to mimic the oral environment *in vitro*.

Method:

Ten disc-shaped specimens were made from each of four different resin composites (Filtek Z250, Voco x-tra base, Beautifil Flow Plus, Beautifil II). Baseline color value of each sample was measured using a spectrophotometer. Each composite was respectively immersed in coffee, an orange/pomegranate juice mixture, black tea, and a mouth rinse on the same day to mimic daily liquid consumption of individuals. Color measurements were taken after 1, 14, and 30 days by spectrophotometer and color change values were calculated. Statistical analyses were executed by one-way ANOVA/Tukey HSD and repeated-measures ANOVA.

Results:

All materials showed significant color change after 1, 14, and 30 days ($P < 0.01$) of immersion in liquids, with the lowest color alteration observed at the 1st day and the highest observed after the 30th day. Among the materials tested, at each time point (1, 14, and 30 days), the lowest color alteration was detected in Filtek Z250 and the highest color alteration was detected in Beautifil II.

Conclusion:

Color alteration of composite resins is affected by composite type and storage time. With the exception of 1 day of storage, color changes of all materials were substantial and clinically unacceptable.

Keywords: Beverage type, Color alteration, Color measurement, Composite resin, Oral simulation, Storage time.

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INTRODUCTION

Composite resins are used in direct/indirect restorations and for the intraoral repair of porcelain restorations to modify the tooth contour and color. The success of an esthetic restoration bases primarily on the color consistency and on the color stability of the material [1].

Staining or discoloration of the restorative material is one of the reasons for replacement of composite restorations, which occurs because of the aging process in the oral environment [2] induced by several extrinsic or intrinsic factors. Extrinsic factors can differ according to the individual's nutrition, and smoking habits [3 - 6]. Intrinsic factors include discoloration of the resin material and depend on the resin matrix, filler weight, particle size distribution, and type of photoinitiator [7, 8].

In the literature the effect of beverage type [4, 5, 9] and storage time [10, 11] on the color stability of restorative materials was investigated. Some studies [12 - 15] investigated the color stability of different restorative materials by testing them both in artificial saliva and beverages in different periods to mimic the oral environment *in vitro*. In these studies [12 - 15] the color change evaluation of composite after each beverage immersion was determined separately from other beverages. Therefore, to more accurately mimic the oral environment *in vitro*, which is naturally exposed to various types of substances throughout the day, in the current study, we investigated the color stability over 30 days of four typical restorative materials that were exposed to multiple types of liquids on the same day. The tested null hypotheses were that (1) the type of composite resin does not affect the color stability of the restorative material, and (2) the overall exposure time in the liquids (1, 14, or 30 days) does not affect the color stability of the composite restorative material.

MATERIALS AND METHODOLOGY

Composite Specimen Preparation

Prior to carrying out the experiment, a power analysis was performed to identify the sample number required to accurately detect significant effects. Based on the power analysis, 10 circular-shaped samples (diameter: 10 mm, thickness: 2 mm) were prepared from each of four composite resins in A2 shade using a cylindrical teflon mold: Filtek Z250 (3M ESPE; St. Paul, MN, USA), Voco x-tra Base (Voco GmbH; Cuxhaven, Germany), Beautifil II (Shofu Inc.; Kyoto, Japan), and Beautifil Flow Plus (Shofu Inc.; Kyoto, Japan). The compositions of the restorative materials used are provided in Table 1. In the Voco x-tra Base group, the composite was injected directly in the mold, whereas the other composites were prepared according to the incremental technique. The last portion of the composite was covered with a glass to get a plane surface. Then, the composites were light polymerized for 20 s from the upper and lower surfaces using an LED light-curing unit (Elipar S10; 3M ESPE; St. Paul, MN, USA) at a light intensity of 1200 mW/cm² and a wavelength of 430-480 nm. During the polymerization, the tip of the light-curing device was positioned perpendicular to the sample surface. After polymerization, the upper surfaces of all samples were finished with medium, fine, and super-fine discs (Sof-Lex, 3M ESPE; St. Paul, MN, USA) for 30 s using a slow-speed hand piece. After each polishing step, each polishing disc was discarded and the samples were thoroughly rinsed with water for 10 s and air-dried for 5 s. All samples were kept in distilled water at 37°C for 24 h in an incubator (Star Dental 320S; İstanbul, Turkey).

Table 1. Contents of the resin composite materials.

| Resin composite | Classification | Filler | | | Resin Matrix | Shade/Lot Number |
|---|---|--|------------|-----------|------------------------------|------------------|
| | | Type | Volume (%) | Size | | |
| Filtek Z250 (3M Espe, St Paul, MN, USA) | Minifilled hybrid | Zirconia/Silica | 60 | 0.6 µm | Bis-EMA, UDMA, Bis-GMA | A2/N439013 |
| Voco x-tra Base (Voco GmbH, Cuxhaven, Germany) | Flowable Bulk-Fill | Silica | 58 | | Bis-EMA, MMA | A2/1335117 |
| Beautifil Flow Plus (Shofu Inc., Kyoto, Japan) | Fluoride-releasing flowable hybrid composite | Multi-functional glass filler, S-PRG filler based on fluoroboroaluminosilicate glass | 47 | 0.8 µm | Bis-GMA, TEGDMA | A2/071350 |

(Table 1) contd....

| Resin composite | Classification | Filler | | | Resin Matrix | Shade/Lot Number |
|--|--|---|------------|-------------|-----------------|------------------|
| | | Type | Volume (%) | Size | | |
| Beautiful II (Shofu Inc., Kyoto, Japan) | Fluoride releasing nano-hybrid composite | Multi-Functional glass filler and S-PRG filler based on fluoroboroaluminosilicate glass | 68.6 | 0.8 μ m | Bis-GMA, TEGDMA | A2/111268 |

Bis-EMA: Ethoxylated bisphenol-A glycol dimethacrylate; UDMA: urethane dimethacrylate; Bis-GMA: Bisphenol A glycol dimethacrylate; MMA: methylmethacrylate; TEGDMA: Triethylene glycol dimethacrylate; S-PRG: Surface pre-reacted glass.

Immersion of Specimens in Liquids

To mimic the daily consumption of a person, after 24 h of incubation, each of the composite resin specimens (n = 10 per group) was individually immersed in flasks containing 5 mL coffee (Nescafe Classic, Nestle Suisse, Vevey, Switzerland) (pH 5.45) at 9:00 for 15 min, 5 mL of a juice mixture (2.5 mL orange juice and 2.5 mL pomegranate juice) (Cappy, Bursa, Turkey) (pH 3.84) at 12:00 for 15 min, 5 mL black tea (Lipton, Unilever, İstanbul, Turkey) (pH 6.04) at 15:00 for 15 min, and 5 mL of an alcohol-free mouth rinse (Listerine Total Care Zero, Johnson and Johnson, İstanbul, Turkey) (pH 4.48) at 18:00 for 30 s, respectively, on the same day. After immersion in each beverage, the samples were rinsed with distilled water and then were kept in artificial saliva at 37°C in an incubator to mimic human oral conditions until the point of immersion in the subsequent liquid. This procedure was repeated for 30 days and each liquid was renewed every day. During the experiment, the flasks were covered to prevent evaporation of the solutions.

The artificial saliva was prepared in the Biochemistry Department of İstanbul University (İstanbul, Turkey) using the formula suggested by Shannon [16]. The composition of the artificial saliva was 4.2 mg/L NaF, 1280 mg/L NaCl, 166.49 mg/L CaCl₂, 125 mg/L MgCl₂.6H₂O, 44.74 mg/L KCl, 7.5 mg/L CH₃COOK, 386 mg/L K₃PO₄.3H₂O, 0.05 mg/L H₃PO₄ (85%) (pH 7).

Color Measurement

Before immersion in the beverages, the initial color measurements of all samples were performed using a spectrophotometer (Vita Easyshade Advance, VITA Zahnfabrik, Bad Säckingen, Germany). Subsequent color measurements were taken at the ends of days 1, 14, and 30 of immersion in the liquids.

Before the measurements, the spectrophotometer was calibrated according to the manufacturer's instructions. Each specimen was removed from the artificial saliva, rinsed with distilled water, and dried with an absorbent paper. Three measurements were conducted at the center of each specimen against a white background and the mean value was calculated. Color alterations were determined using the Commission Internationale d'Eclairage L*a*b* color system (CIE L*a*b*). The CIE L*a*b* color system is a three-dimensional color measurement system, where L* is the lightness coordinate, and a* and b* are the chromacity coordinates in the red-green axis and the yellow-blue axis, respectively [17]. Color alteration values (ΔE) between initial and at the end of 1, 14, and 30 days were computed from the mean ΔL , Δa , and Δb values for each sample with the subsequent formula [1, 18]:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

According to this formula, ΔL , Δa , and Δb are the variations in the L, a, and b values, respectively, at baseline and after immersion at each time interval (1, 14, and 30 days).

To determine the relationship between the amount of color alteration recorded on a spectrophotometer to the clinical environment, data were converted to the National Bureau of Standards (NBS) system (Table 2) [19, 20]. According to this system, ΔE values can be described by the subsequent equation [19, 20]: NBS unit = $\Delta E \times 0.92$.

Table 2. National Bureau of Standards (NBS) system of expressing color differences [19,20].

| ΔE | NBS Criteria |
|------------|--------------------------------------|
| 0–0.5 | Trace: Remarkably slight alteration |
| 0.5–1.5 | Slight: slight alteration |
| 1.5–3 | Noticeable: Observable alteration |
| 3–6 | Appreciable: Apparent alteration |
| 6–12 | Much: Remarkably apparent alteration |
| 12 or more | Very much: Alteration to other color |

NBS unit = $\Delta E \times 0.92$

Statistical Analysis

Statistical analyses were done using SPSS version 22.0. All data were firstly analyzed by the Shapiro-Wilk test. One-way ANOVA and Tukey HSD tests were done to assess color changes of all materials between days of measurement. Repeated-measures ANOVA and post-hoc Bonferroni tests were executed to compare the color change of each material on different days ($P < 0.05$).

RESULTS

The results of the statistical analyses of color change values are presented in Table 3. According to one-way ANOVA and the Tukey HSD test, after the first day, Beautiful II showed a significantly higher color change than the Filtek Z250 and Voco x-tra Base materials; in addition, Beautiful Flow Plus showed a higher color change than Filtek Z250 ($P < 0.05$). However, no noteworthy color changes were observed between Filtek Z250 and Voco x-tra Base, Voco x-tra Base, and Beautiful Flow Plus, or between Beautiful Flow Plus and Beautiful II. At the 14th day, Beautiful Flow Plus and Beautiful II showed higher color changes than the other materials ($P < 0.05$). Furthermore, Beautiful II showed a higher color change than Beautiful Flow Plus ($P < 0.0001$). However, no important difference was observed between Filtek Z250 and Voco x-tra Base. At the end of the 30th day, Beautiful II showed the highest color change of all materials, and Beautiful Flow Plus and Voco x-tra Base showed higher color changes than Filtek Z250 ($P < 0.0001$). However, no significant color alteration was detected between Beautiful Flow Plus and Voco x-tra Base.

The results of repeated-measures ANOVA showed significant color changes in each material after 1, 14, and 30 days ($P < 0.01$). For each material, the lowest color alteration was detected after the first day, and the highest color alteration was detected after the 30th day of storage.

According to the NBS system (Table 2), slight color change in Filtek Z250, noticeable color change in Beautiful Flow Plus/Voco x-tra Base, and appreciable color change in Beautiful II specimens were observed at the end of the first day. At the end of the 14th day, much color alteration was observed in Filtek Z250 and Voco x-tra Base, and very much color alteration was observed in Beautiful Flow Plus and Beautiful II specimens. At the end of the 30th day, all of the restorative materials showed very much color changes.

Table 3. Mean and standard deviation of color alteration values (ΔE) of restorative materials at 1, 14, and 30 days of daily immersion in various liquids.

| | Mean \pm SD | | | | P^1 |
|---------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|--------------|
| | Filtek Z250 | Voco x-tra Base | Beautiful Flow Plus | Beautiful II | |
| 1 day | 1.17 \pm 0.6 ^{a,A} | 1.81 \pm 1.29 ^{ab,A} | 2.39 \pm 1.04 ^{bc,A} | 3.01 \pm 0.36 ^{cA} | 0.001 |
| 14 days | 7.31 \pm 1.9 ^{a,B} | 8.73 \pm 1.38 ^{ab,B} | 12.59 \pm 1.81 ^{b,B} | 18.05 \pm 3.78 ^{c,B} | 0.001 |
| 30 days | 17.26 \pm 3.97 ^{a,C} | 25.95 \pm 2.56 ^{b,C} | 26.28 \pm 1.73 ^{b,C} | 35.18 \pm 2.65 ^{c,C} | 0.001 |
| P^2 | 0.001 | 0.001 | 0.001 | 0.001 | |

¹One-way ANOVA, ² Repeated-measures ANOVA.

Different small letters in the same rows and different capital letters in the same column show statistical importance ($P < 0.05$).

DISCUSSION

Some studies [21, 22] reported that color change of composite resin stored in water showed highest degree of the color change after 24h and 7 days. On the other hand, Domingos *et al.* [14] reported that the most pronounced color change of composite was occurred after 30 days. In addition Uchimura *et al.* [23] reported that the color change of composite resin was changed up to day 14 and then it was stabilized, therefore they pointed out that 14 days could be used as a reference for color comparison. Based on the results of these studies [14, 21 - 23], in this study color stability of four different resin composites that were immersed in multiple liquids (coffee, orange/pomegranate juice, tea, and mouth rinse respectively) on the same day were investigated after a period of 1, 14, and 30 days. All of the liquids used in the study were selected as colorant agents due to their constant consumption in daily life. To simulate clinical conditions as much as possible, the specimens were immersed in artificial saliva between immersions in each of the liquids.

It was reported that water sorption induces a weaker bond between the resin matrix and filler particles, and the consequent micro-cracks or interfacial gaps produced between the matrix and filler enable for stain penetration and discoloration of composite restorations [4, 7, 10, 24, 25]. Therefore, composite resins that consist of higher amounts of resin matrix [7, 10], larger filler particles [26], and low concentration of filler particles [27] could have an increased

tendency toward discoloration. In addition, the type of resin matrix has also been reported to play an important role in staining susceptibility [28, 29]. Triethylene glycol dimethacrylate (TEGDMA) consists of hydrophilic groups and thus shows a high predisposition to water sorption. Kalachandra *et al.* [24] reported that water uptake in bisphenol A glycol dimethacrylate (Bis-GMA)-originated composite resins increased in proportion with the amount of TEGDMA. On the other hand, ethoxylated bisphenol A glycol dimethacrylate (Bis-EMA) and urethane dimethacrylate (UDMA) are highly hydrophobic, showing low water sorption and solubility characteristics [30].

Similar to the findings of previous studies [4, 7, 10, 25 - 29], the results of this study demonstrated that the restorative material type influenced color changes, supporting the influence of resin matrix composition, amount of filler weight, filler type, and filler particle size on color change susceptibility. Therefore, our results rejected the first null hypothesis because the type of composite resin affected the color stability of restorative material. In this study, the lowest color alteration was detected in Filtek Z250, which is likely due to the fact that it has a more hydrophobic resin matrix structure (Bis EMA, UDMA), higher filler weight (82%), and smaller filler particle size (0.6 μm) than the other materials. On the other hand, at all days, Beautifil Flow Plus and Beautifil II showed higher color changes than Filtek Z250 ($P < 0.05$). This is due likely to the fact that both of these composites consist of a hydrophilic resin matrix type (TEGDMA) and have a higher filler particle size (0.8 μm) than Filtek Z250 (Table 1). In addition, the color difference between Filtek and Voco x-tra Base was only significant after 30 days, whereas the color difference between Beautifil Flow Plus and Beautifil II was significant after 14 days. These results further demonstrate that if the resin matrix of the restorative material consists of hydrophilic components, color alteration may occur earlier.

In contrast, Beautifil II showed higher color alteration than Beautifil Flow Plus. Beautifil II has a higher filler weight than Beautifil Flow Plus, but the two materials have the same filler particle size and hydrophilic resin matrix composition. Therefore, we consider that this difference might be due to the fact that the ytterbium trifluoride in Beautifil II induced more water sorption than Beautifil Flow Plus, which would lead to enhanced color alteration.

Similar to previous studies [11, 31, 32], a spectrophotometer and the CIE L*a*b* coordinate system was used to assess the color alteration due to the advantages of repeatability, sensitivity, objectivity, and determination of small color changes. Several studies [7, 25, 33, 34] reported that ΔE values equal to or greater than 3.3 were considered clinically appreciable and unacceptable. In addition, in this study, NBS criteria were used to determine the relationship between the amount of color alteration recorded on a spectrophotometer and the clinical environment. After 1 day, all of the composite materials showed clinically acceptable color changes. However, after 14 and 30 days, the color alteration of all composite materials was deemed to be unacceptable, with very much color changes ($\Delta E \geq 12$) noted at 30 days.

The second null hypothesis, that the total time of exposure does not have an important effect on the color change of the restorative materials, was also rejected. For each composite type, the lowest color alteration was observed after the 1st day, and the highest color alteration was observed after the 30th day. This finding was also in agreement with previous studies [5, 28, 33, 35]. Although the results of this study showed similarity to these studies [5, 28, 33, 35] results, this study differed from them that it presented the daily consumption of a patient in in vitro conditions and the methodology used was different.

There were some limitations in this study. Although all of the composites were polished with Sof-Lex discs, the effect of surface roughness on the color change of restorative materials was not evaluated. On the other hand, the samples were not brushed after the oral simulation process, which might have influenced the staining susceptibility of the composite resins. In future studies, the influence of surface roughness, brushing, and beverage pH on the color stability of the restorative materials should be investigated.

CONCLUSION

Color alteration of composite resins was influenced by the sort of composite (resin matrix type, filler weight, filler particle size, and filler type) and storage time.

With the exception of the first day of storage, the color changes of all the materials were clinically unacceptable.

Among the materials tested, Filtek Z250 showed the lowest color alteration and Beautifil II showed the highest color alteration.

In clinical practice when selecting an appropriate restorative material, the dentists should consider the drinking and oral hygiene habits of the patients and the factors that may affect the color stability of restorative materials. In addition, patients should also be informed about the staining potential of the restorations.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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